Cell-Free DNA Kit

Catalogue No.:abx098870

Abbexa's Cell-Free DNA Kit is an ideal solution for lysing samples by enzyme hydrolysis and purifying cell-free DNA by specific adsorption of silica magnetic beads. It is suitable for isolating and purifyling high quality cell-free DNA from 0.5-10 ml serum or plasma. The extracted DNA can be used in PCR, qPCR and NGS, and also magnetic beads-based nucleic acid extraction systems.

Kit Components:

- Binding Buffer: 120 ml
- Clean Buffer: 30 ml
- Wash Buffer: 24 ml
- Elution Buffer: 4 ml
- Proteinase K (20 mg/ml): 3 × 1 ml
- 20% SDS: 6 ml
- Magnetic Cell-Free Beads: 2 ml

Material Required But Not Provided:

- Magnetic Stand (with tube holder)
- Vortex Mixer
- Centrifuge and Microcentrifuge Tubes
- Pipettes and Pipette Tips
- Isopropyl Alcohol

Target:	Cell-Free DNA
Storage:	Store magnetic beads at 2-8 °C for up to one year, do not freeze. Store all other reagents at room temperature.
Note:	THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC, THERAPEUTIC OR COSMETIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION.

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Directions for use:

Reagent Preparation:

• **Working Binding Buffer:** Add 40 ml isopropyl alcohol to 120 ml Binding Buffer to prepare the Working Binding Buffer solution.

• **Working Clean Buffer:** Add 30 ml isopropyl alcohol to 30 ml Clean Buffer to prepare the Working Clean Buffer solution.

• **Working Wash Buffer:** Add 96 ml isopropyl alcohol to 24 ml Wash Buffer to prepare the Working Wash Buffer solution.

Notes:

• Vortex magnetic beads before use.

• Use germ-free, nucleic-acid-free and nuclease-free centrifuge tubes and pipette tips.

• The component content of cell-free DNA is extremely low, we recommend using low nucleic acid adsorption centrifugal tubes for plasma storage, DNA extraction and DNA storage.

• Avoid repeated freeze/thaw cycles of reagents, samples and isolated DNA.

Reaction System:

Prepare the reaction system in a 15 ml or 50 ml centrifuge tube according to the table below.

Component	**Plasma Volume**				
	0.5 ml	1 ml	2 ml	4 ml	10 ml
20% SDS	25 µl	50 µl	100 µl	200 µl	500 µl
Proteinase K	15 µl	30 µl	60 µl	120 µl	300 µl
Working Binding Buffer	0.75 ml	1.5 ml	3 ml	6 ml	15 ml
Magnetic Cell-Free Beads	10 µl	20 µl	40 µl	80 µl	200 µl

Assay Procedure:

1. Set up a reaction system according to the table above.
2. Vortex the tubes for 15 seconds, then leave at room temperature for 20 minutes. Invert the tube 3-5 times throughout this time period.
3. Magnetic separation: Place the centrifuge tubes on a magnetic stand, then gently spin the tubes left and right by hand. Reverse the spin direction when the magnetic beads begin to aggregate towards the tube wall, and repeat 2-3 times. Ensure that any beads on the lid aggregate to the tube wall. Allow to stand for 2 minutes and ensure all beads are aggregated to the tube wall.
4. Discard the supernatant from the opposite side of the magnetic beads, taking care not to remove the beads themselves. Take the tubes off the magnetic stand and add 1 ml of Working Clean Buffer (with isopropyl alcohol). Vortex for 15 seconds, then transfer to a new 1.5 ml centrifuge tube. If magnetic beads remain in the original tube, transfer the supernatant back to the original tube, wash, then transfer to the 1.5 ml centrifuge tube. Repeat Step 3 to carry out another magnetic separation.
5. Discard the supernatant. Take the tubes off the magnetic stand and add 1 ml of Working Wash Buffer solution (with isopropyl alcohol). Vortex for 15 seconds, then repeat Step 3 to carry out another magnetic separation.
6. Repeat Step 5.
7. Discard the supernatant, including any liquid on the lid. It is recommended to use smaller size pipette tips to remove the supernatant thoroughly.
8. Allow to stand and air-dry for 5-10 minutes.
9. Add Elution Buffer according to the table below

Component	**Original Plasma Volume**				
	0.5 ml	1 ml	2 ml	4 ml	10 ml
Elution Buffer	20 µl	30 µl	50 µl	75 µl	200 µl

Vortex the tubes for 5 minutes.

10. Place the centrifuge tubes on the magnetic stand. Transfer the liquid into a new 1.5 ml low nucleic acid adsorption centrifuge tube, taking care not to remove the beads themselves.
11. Store the isolated DNA at -20 °C.

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